

Procedure Flowchart

Design PCR primers based on DNA sequence of reference strain or individual

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Mix genomic DNAs of the reference and test strains

PCR-amplify DNA regions across the genome, either single reaction or multiplexed reactions

Combining single and/or multiplexed PCR reactions

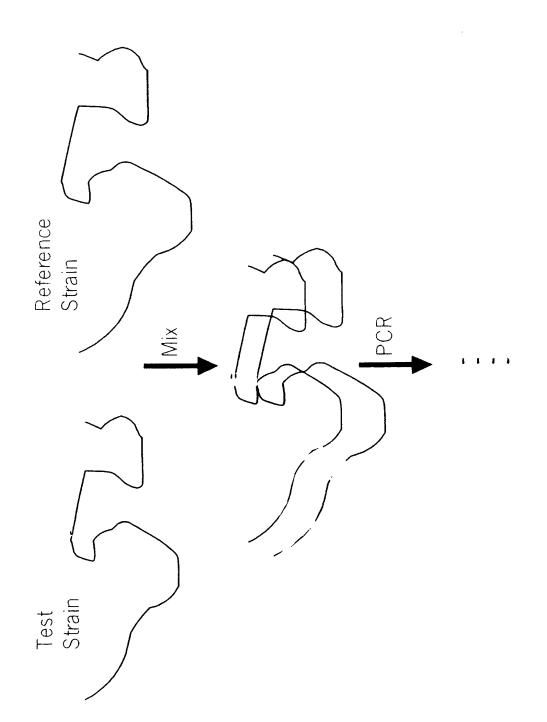
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Denature and anneal PCR products to form heteroduplexes/homoduplexes

Scan by temperature gradient electrophoresis to identify heterozygous DNA fragments/variants with a single SNP/simple Indel formed by mixing reference and test strains

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Determine sequences of variants to locate the exact positions of the SNP/Indel



Homoduplex heteroduplex formation

omozygote

-GATGAGGCATTTGAC--CTACTCCGTAAACTG

eterozygot

--GATGAGGCATTTGAC--CTACTCCGTAAACTG-

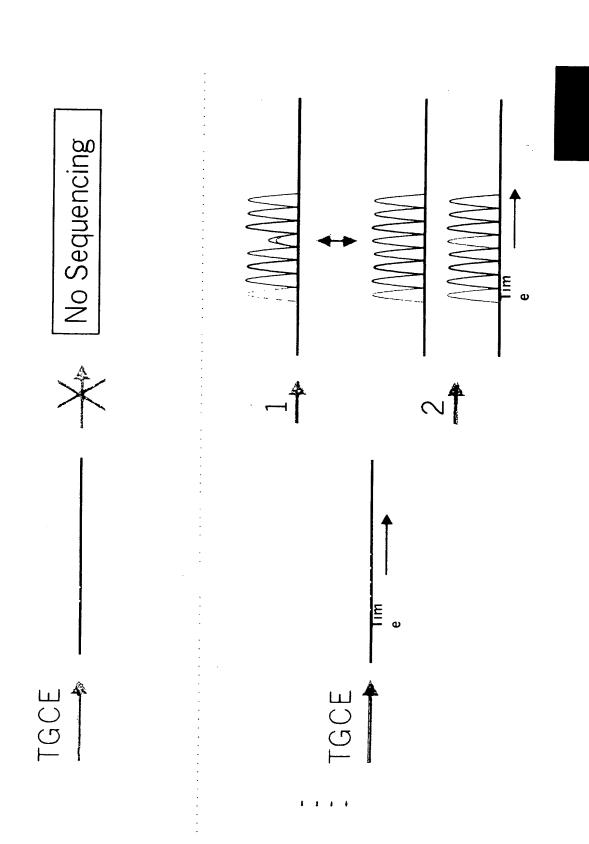
-GATGAGGCGTTTGAC - CTACTCCGCAAACTG

Denature, a 三元の

CTACTCCG AAACTG -GATGAGGCGTTTGAC--GATGAGGCETTTGAC--GATGAGGCGTTTGAC--CTACTCCGCAAACTG-

-GATGAGGCATTTGAC-

CTACTCCGT AAACTG -CTACTCCGTAAACTG



Sample pooling

